NCI ETI Branch
Flow Cytometry Core Laboratory

Using the LSR II.

The LSR II is our newest benchtop flow cytometer, equipped with four lasers and capable of up to ten color analysis. It is located in **Building 10 room 12C216**.

If you have never used this instrument before (even if you are familiar with other BD instrumentation), you ***must*** contact Veena Kapoor (5-6378, veenak@helix.nih.gov) or Bill Telford (5-6379, telfordw@mail.nih.gov) prior to use. These instruments are located in space shared by investigators employed by the Flow Lab; please be respectful of the people who live and work there.

Scheduling time and training on the LSR II. The LSR II can be used by ETI Branch faculty members or investigators *who have received the appropriate training*. If you have never used this sytem before, please set up an appointment with Veena Kapoor for instrument instruction. Training will take approximately two hours. ***Please do not attempt to use this instrument without training.***

Sign up for the LSR II on the calendar located on the instruments. Include your name, your PI's name, phone number and the time period you require for analysis. Alternately, call or e-mail Veena or Bill to reserve time. Sign up *only for time you really need*; if you need to cancel a scheduled run, visit, call or e-mail the Core to remove your name from the sign-up sheet. Allow sufficient time for instrument start-up (about ten minutes) and post-experiment instrument cleaning and data backup (another ten minutes) when calculating your estimated usage time.

Instrument care and maintenance. Follow the start-up and shut-down instructions (posted on the front of the instrument). Since the LSR II has a more complex laser and optical bench configuration than the FACSCalibur or FACScan, we run a daily QC check with fluorescent beads; check will Bill or Veena to see if this has been done for the day. When you have completed your experiment, remember to flush the instrument with 10% bleach and distilled water according to the posted directions. Each user should empty the waste tank and refill the sheath after use. Sheath fluid is provided by the Core. These directions **must** be followed, no matter how small your experiment is. Following these simple maintenance instructions will greatly enhance day-to-day instrument performance and minimize downtime for servicing. Repeated failure to take care of the instrument in this manner will necessitate frequent repair of the fluidic system, with resultant instrument downtime. Laboratories who repeatedly fail to properly maintain the instrument may lose usage privileges. If you have any questions about instrument maintenance, please ask! If you notice any problems with instrument operations, please let Veena or Bill know ASAP!

Data analysis. The LSR II is a fully digital instrument and uses a Windows NT-based data acquisition software package. The data is stored in a database format that is not immediately compatible with CellQuest, WinMDI or other flow analysis packages. For data analysis by other programs, you must convert your files to .FCS format. When you have completed your experiment, use the mouse to highlight the sample files you wish to convert, then go to the File / Export menu. The parameters saved during data acquisition will then be displayed (FSC, SSC, FL1, etc.) Click on the ones you wish to convert, then press EXPORT. The files will then be moved to the c:/export folder on the hard drive in . FCS format. These can then be analyzed directly by PC-based flow cytometry programs such as WinMDI. For analysis by Mac programs such as FlowJo or CellQuest, transfer the files to a PC disk, move then to your Mac hard drive, then use the program FACSConvert to convert them to Mac format.

Since data conversion and backup on the LSR II is somewhat complicated, get Bill's or Veena's help the first time. As with the other benchtop instruments, it is important for each user to back up their own data in this fashion. We will back up the DigiFACS databases once a month; however, we will not do backups of everyone's converted data. ***YOU are primarily responsible for the backup of your own data!***

Monday mornings from 10:00 to 12 noon will be reserved by the facility for routine instrument preventative maintenance. Please do not plan to use the instrument during this time.

This material is prepared by the Telford Lab for the NCI ETI Branch and its friends. Revised 11-11-02.